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MOLECULAR BIOLOGICAL TECHNIQUES RELEVANT TO THE STUDY OF THE OCEAN: A PERSPECTIVE FROM EUROPE

a report

by K.E. Cooksey

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This report covers research that promises to increase our understanding at the molecular level of biological processes in the marine environment. Included is some information that was not derived from the study of marine organisms. In these cases, either the principles demonstrated or the techniques used could be translated for use in studying a marine problem. Although the research reported here is at the molecular level, it does not imply that traditional marine science is being abandoned in Europe. An annual meeting, sponsored by the European Marine Biological Symposium and held at a major marine science center, focuses on whole macroorganism biology and ecology.

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This special issue of ESNIB has been compiled by Dr. Keith E. Cooksey. Dr. Cooksey was the Liaison Scientist for Biochemistry, Microbiology, and Marine Biotechnology in Europe and the Middle East for the Office of Naval Research European Office.

MOLECULAR BIOLOGICAL TECHNIQUES RELEVANT TO THE STUDY OF THE OCEAN: A PERSPECTIVE FROM EUROPE . . . . . . K.E. Cooksey

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Molecular Biological Techniques Relevant to the Study of the Ocean: A Perspective from Europe

by Keith E. Cooksey, the Liaison Scientist for Biochemistry, Microbiology, and Marine Biotechnology in Europe and the Middle East. Dr. Cooksey was on leave from Department of Microbiology, Montana State University, Bozeman, Montana, where he is Professor of Microbiology.

Executive Summary

Introduction

My predecessor at the Office of Naval Research European Office (ONR Europe), Dr. Claire E. Zomzely-Neurath, summarized in a general way most aspects of biotechnology in Europe and the Middle East (see ESNIB 89:06). To cover the same ground would have been superfluous. In any case, a broadly based description of the field is probably only warranted every 5 years or so. The goal of my liaison tour therefore was to complement Dr. Neurath's report.

The Assessment System for European Technology and Science (ASSETS) database was used to define this complementary approach. Based on an ASSETS search, the Office of Naval Research (ONR) interests, and my own knowledge, my report focuses on science that promises to extend our understanding of the processes at the molecular level that define and control the biological workings of the ocean. Not all the science that is reported is classically marine. However, if I could see that the techniques involved were adaptable usefully to the marine environment, I included them. Much of what I report has been defined by the current programs and potential future interests of scientists in ONR, the Naval Research Laboratory (Washington, D.C.), David Taylor Research Center (Annapolis and Bethesda, Maryland), and the Naval Oceanographic and Atmospheric Research Laboratory (Bay St. Louis, Mississippi). Table 1 shows those aspects of the report that I consider to impact most on Navy research and development.

Description of Research Programs

The University of Stirling, Scotland, houses the Natural Environment Research Council (NERC, U.K.) unit for Marine Biochemistry. The focus is in aquacultural and biochemical aspects of marine lipids and molecular toxicology. Researchers are developing fish cell cultures for toxicological testing as models alternative to whole fish. When whole fish are used, death of the fish is the response usually measured. However, in tissue culture experiments, molecular lesions are detected, for instance, by probes, antibodies, or enzyme assays.

Plymouth Marine Laboratory, England, is a new organization formed from the Plymouth Marine Biological Laboratory and the newer NERC Marine Environmental Laboratory. The combined institute has considerable expertise in running analytical flow cytometers at sea. The laboratory also is involved in fish cell culture and has spent considerable money for molecular biological equipment to move more rapidly into this area of science. Another forté is designing and operating towed arrays carrying multiple oceanographic chemical and physical sensors.

The Station Marine de Roscoff houses the main French group working on thermal vent microorganisms. I arranged for the principal investigator, Dr. Prieur, to visit the U.S. to organize collaborative cruises with ONR contractors using a French submersible. Other aspects of this laboratory include fundamental studies of cellular division and macroalgal biotechnology.

The Laboratory for Materia Technica in the Dental School of the University of Groninengen, the Netherlands, leads in the image analysis of the kinetics of cellular adhesion to surfaces. Researchers here measure cooperativity in adhesion; i.e., the influence of one cell on the adhesion of another. They have also done definitive work on the influence of the so-called conditioning film on cellular adhesion and how the surface chemistry of the clean substratum is involved in this phenomenon.

The laboratory of the University of Aarhus, Denmark, is the driving force behind the recent explosion in the use of microelectrodes in microbial ecology. They have several new sensors in development.

The Menai Bridge Marine Laboratory, University of Wales, is redirecting some of its resources towards establishing a molecular marine science program by appointing two new faculty members--in microbiology and symbiosis, respectively. The research of the Professor of Ocean Science, Dr. P.J. LeB. Williams is one aspect of the laboratory that is well known. His work sheds new light on microscale patchiness in the ocean.

Many of the programs at Sweden's University of Gothenburg's Department of General and Marine Microbiology are similar to those supported in the U.S. by ONR. Notably, the research on differentiation in nonsporulating bacteria and that on biocorrosion. Using marine microorganisms for a change (rather than *Escherichia coli*), it has been shown that starving bacteria in the ocean are involved in biogeochemical cycling. The bacteria are not dormant. Another seemingly contradictive finding is that some types of microorganisms protect against corrosion; they do not cause it.

None of the work at the Swiss Federal Institute of Technology (ETH), Dübendorf, is marine; in fact, most of it is carried out as fundamental underpinning to wastewater treatment. The results, however, are widely applicable in microbial ecology and biotechnology. Research has cast doubt on two of the basic tenets of microbiology. In the first, microbiologists and engineers at ETH have shown that at very low substrate concentrations (i.e., environmentally relevant concentrations), microorganisms utilize multiple substrates simultaneously (i.e., diauxie does not take place). A second finding is that many of the organisms placed in the genus *Pseudomonas* have probably been put there incorrectly. In reality, the well-accepted idea that pseudomonads are metabolically diverse may not be true.

A visit to the Kiel Institut für Meereskunde, Federal Republic of Germany, revealed an emphasis on deep sea sediment microbiology and measurements of marine microbiological activity in general. One laboratory was working on an automated device to measure *in situ* microbiological activity in the deep sea.

Several visits to Europe and the Middle East were postponed or canceled because of the Persian Gulf crisis or other armed conflicts.

Table 1. Molecular Marine Biological Science

Location	Highlights
Groningen, the Netherlands	Hydrodynamics/microbial attachment "Shining through" phenonemon
Kiel, Federal Republic of Germany	Sediment microbiology Deep sea microbiology
Gothenburg, Sweden	Differentation in nonsporulating bacteria Corrosion protection
Menai Bridge, Wales	Biogeochemical ocean flux Molecular biology of marine organisms
British Antarctic Survey	Krill biology
Stirling, Scotland	Lipids in fish aquaculture Molecular toxicology
Plymouth, England	Chemoreception Towed sensor arrays Fish cell culture for toxicology
Roscoff, France	Macroalgal biotechnology, chemoreceptional Molecular biology of thermal vent organisms
Aarhus, Denmark	Microsensor technology
ETH, Switzerland	Pseudomonads have no diverse physiologies Mixed substrates: simultaneous use

Assessment

The involvement of molecular biological techniques in marine and environmental biology in general is less well developed in Europe and the Middle East than in the U.S. The Europeans recognize this; they are making a concerted effort to redress the situation. One of the primary goals of the newly formed Marine Research Stations Network of the European Community (EC) is to hold courses rather similar to those held at several U.S. marine institutions. ONR Europe has provided information concerning the U.S. offerings to the organizers. Like their U.S. counterparts, the courses will teach the basics of molecular biological techniques to marine scientists. The first course will be held at Plymouth, England.

Since molecular and cellular biology in general in Europe is just as advanced as that in the U.S., it is interesting to speculate why the involvement of these types of science in marine research is less well developed there than the U.S. My view is that research centers where traditional marine science and cutting-edge biology existed in parallel acted as catalysts in the development of marine molecular biological science. This is not to say however, that we can ignore developments in Europe. Listed below are places where I believe continued liaison is appropriate and where collaborations should be encouraged.

Anyone seriously considering using microelectrodes for microbial ecological measurements should visit, or better, collaborate with Dr. N-P. Revsbeck's group at the University of Aarhus, Denmark. They are the leaders in new electrode chemistries, as well as, the most knowledgeable group on electrode manufacture.

The Plymouth Marine Laboratory and that at Menai Bridge are involved in programs (e.g., BOFS) concerned with biogeochemical cycling and its relation to global climatic change. They have been successful in designing equipment for underwater measurements from oceanographic vessels. I know they welcome collaborations with their U.S. counterparts in the study of global change. This should be encouraged.

Close contact should be maintained with the Station Biologique de Roscoff since several of the programs there are similar to those promoted by ONR in the U.S. Leaders of laboratories in various parts of the U.S. now have research agreements or proposed joint research cruises with scientists in the laboratory, so I expect this recommendation will be followed.

The Dental School, Technical Materials Laboratory at Groningen, the Netherlands, should be regarded as the most important unit dealing with biology at interfaces in terms of ONR's interests.

The molecular approach to eco-toxicology is well developed at the University of Stirling, Scotland. The work here compliments the program in bioremediation followed by ONR. This group looks at bio-effects of anthrogenic chemicals in the environment, whereas, ONR's program is concerned in removing the compounds that cause the effects. The research here has wider significance to the U.S. Department of Defense (DOD) since bioremediation is targeted as a technology critical to its operations in the 1990's.

The Department of General and Marine Microbiology at the University of Gothenburg, Sweden, is a leader in the physiology of marine microorganisms on surfaces and their role in corrosion. I consider that continued liaison with this group whose research will impact on future ONR and other U.S. programs is appropriate. Interests in biomining for the recovery of strategic metals should follow the research of Dr. K. Pedersen. Dr. Karin Malmkrona will visit the U.S. in Spring 1992 to establish U.S. - Swedish scientific links, perhaps with the support of ONR Europe.

The research being carried out at the Swiss Federal Institute for Water Resources and Water Pollution Control, Dübendorf, should continue to be monitored by ONR since they are innovative and work successfully at the very difficult boundary between engineering and microbiology. Their work is relevant in basic marine microbiology and particularly so in the area of bioremediation. The laboratory would be a particularly useful venue for a post doctoral engineer or microbiologist to learn about the interface between the two disciplines.

Molecular Biological Techniques Relevant to the Study of the Ocean: A Perspective from Europe

Comprehensive Introduction

This report covers research that promises to increase our understanding at the molecular level of biological processes in the marine environment. I have included some information that was not derived from the study of marine organisms. In these cases, either the principles demonstrated or the techniques used could be translated for use in studying a marine problem.

Like most assessments of science in these pages, this one does not pretend to be global. It was impossible to visit everywhere I wished during my tenure at Office of Naval Research European Office (ONR Europe). For instance because of the Persian Gulf crisis, a scientific meeting in Italy on the microbiology of the Mediterranean Sea was postponed. Similarly, trips to the Middle East and Yugoslavia were curtailed. Nevertheless, I surveyed a major marine science center in most of the European countries in the forefront of this discipline. I report on them below in no particular order.

General Remarks

Although most workers at marine centers I visited were completely open about their research, some at several other types of laboratories were more guarded, especially in the United Kingdom (U.K.). The British Department of Trade and Industry operates a funding program for joint university-industry research undertakings (called LINK). The program is administered through the research councils; e.g., Science and Engineering Research (SERC). The aim is to increase the benefits *to the U.K. economy* from government-funded research (my emphasis). Because the research is funded up to 50 percent by industry and the government, respectively, the research results often are available only to these participants. Usually, intellectual property rights rest with the industrial partner. Areas of marine science where programs of this type have hindered communication are in biofouling research and the production of novel polysaccharides.

Although the research reported here is at the molecular level, it does not imply that traditional marine science is being abandoned in Europe. An annual meeting, sponsored by the European Marine Biological Symposium and held at a major marine science center, focuses on whole macroorganism biology and ecology.

As part of my liaison activity at ONR Europe, I have been involved in the interaction between the U.S. National Association of Marine Laboratories (NAML) and the European Marine Research Station (MARS) Network. One of the major objectives of the MARS Network is to promote the use of molecular biological techniques in understanding the ocean. To this end, they will hold courses in molecular biological techniques in 1992 at the Plymouth Marine Laboratory (PML). The ONR Europe supplied names of potential instructors for the course, as well as descriptions of courses run at Woods Hole, Massachusetts, and the University of Southern California. The PML is a recognized center for using flow cytometers in studying the marine particulate fraction. Therefore, I expect this subject to be covered as well as the use of immuno- and DNA-based probes. The increasing level of interest in this area of science was evident also from the number of people who attended two sessions of the 4th Marine Microbiology Symposium held in Kiel, the Federal Republic of Germany (FRG), in 1990. In one session, Dr. M.G. Höfle, Max Planck Institute for Limnology, Plön, FRG, presented a lecture covering molecular approaches to marine microbiology. Some of the work described was supported by the Office of Naval Research (ONR) (Edward De Long, Norman Pace) or the National Science Foundation (NSF) (David Ward). The other session was a workshop during which it became obvious there is considerable disagreement how comparatively simple radioactive procedures to measure growth (e.g., uptake of ^{3}H-thymidine) should be interpreted. This does not augur well for the successful adoption of relatively sophisticated multistep techniques dependent on extracting vanishingly small amounts of nucleic acid from environmental samples (see *ESNIB* 91-02:35-36).

Denmark

The Institute for Genetics and Ecology at the University of Aarhus

Introduction

The Institute for Genetics and Ecology at the University of Aarhus is best known for the advances its faculty has made in microsensor technology. Using these sensors, Drs. Niels-Pieter Revsbeck, Bo Jorgensen, and Tom Blackburn (together with other investigators and students) have shown the existence of steep chemical spatial gradients in biofilms and aquatic sediments. Five years ago, only pH, sulfide, and dissolved oxygen electrodes were available, but recently a combined O_2/N_2O electrode has been developed. Now a nitrate sensor is available. This suite of electrodes allows basic aerobic, photosynthetic, and anaerobic metabolism to be measured as a function of depth in a biofilm.

Microsensor Use

I asked graduate student Tage Dahlsgaard about the accumulation of chemical species/depth profile information. Specifically, I wanted to learn of the potential for disruption of the profile by puncturing the biofilm biomass with a microsensor about 10 μm in diameter. He told me that profiles entering the film and leaving it were similar, provided the withdrawal was done slowly. Most problems in this area were caused by the micromanipulator carrying the microsensor, rather than by the sensor itself. The use of stepping motors to move the sensors rather than manually is helpful in overcoming this problem. Nevertheless, apparently a sharp, pointed electrode gives a more reproducible response than a blunt one!

New Sensors

I was very interested to learn of three new sensors. The first of these measures in a two stage process both N_2O and O_2, thus allowing denitrification profiles to be calculated.

A second is yet undescribed outside the laboratory; it is an ion exchange electrode that is specific for NO_3^-. Unfortunately, this specificity is not as high as one would like. There are serious interferences by HCO_3^- and Cl^-, making the electrode of no use in marine systems.

The third sensor is really only a fiber optic light pipe. However, this group has developed a highly sensitive collector and mated to it a diode array spectroradiometer so photosynthetic action spectra can be measured as a function of depth in a sediment or cellular film. The system has a spatial resolution of 50 μm and a response time of 33 msec. Spectra collected with this time constant are smoothed by a computer. One of the major findings with this device has been that in siliceous sediments, the light just below the surface is up to twice the incident radiation. The reason for this is that light on a surface is measured using a 2π collector, whereas this sensor uses a spherical collector which approximates to 4π geometry. Nevertheless, a photosynthetic organism just below the surface could receive as many photons as an organism on the surface of a sand sediment. The thesis topic for Mr. Talgaard involves modelling the spectral penetration by light of sediments of various grain sizes and composition.

Concluding Remarks

This department has a production line to prepare sensors of all the types mentioned--very important if one considers their fragility. There is no better place to learn this type of technology, which is so important in *situ* measurements for microbial ecology.

Federal Republic of Germany

Institut für Meereskunde of the University Kiel

Introduction

The Institut für Meereskunde of the University Kiel (Institute) can trace its scientific lineage to the 1600's when a university geology professor measured temperature and salinity in local waters. However, the current organizational entity, which is a part of the University of Kiel (Christian Albrechts Universität), was not founded until 1937. Today, the Institute stands on the Kiel fiord at the southwestern end of the Kiel Bight which in turn opens to the southern margin of the Baltic Sea. Deep water allows major oceanographic vessels to moor at the rear of the main building within a few meters of the laboratories. The provost, Professor Kortum, told me that the majority of the Institute's core budget comes equally from either the Federal government (Federal Ministry of Research and Technology) or the local government (State of Schleswig-Holstein). The current budget is about DM 32 million per year with approximately another 10 percent from project funding. This contrasts sharply with U.S. institutions where project funding is usually by far the larger proportion of the total budget.

The Institute has approximately 500 staff (including 100 scientists) and about 300 students. There is a policy to employ young scientists on contracts of limited duration. Hoping to create flexibility, the goal is to have about one third of all positions of this type. The overall foci of the Institute are: processes governing global change, marine food chains, and environmental protection.

While at Kiel, I saw the new research vessel the F.S. Alkor which replaces a ship of the same name. The new ship is four times larger (1,000 tons) and nearly twice as long (55m) as the old one. It has highly adaptable space and the dry laboratories are as good as some of the best I have ever seen on land. Although this ship is much longer than the old Alkor, it still carries the same number of scientists (12).

The Institute has ten departments, but I visited only the scientists in marine microbiology. Other departments are similar in name to those in any major marine laboratory (i.e., Fisheries, Physical, Chemical Oceanography). I found the inclusion of microbiology as a specific department most unusual, since it is not part of Marine Botany or even of an all-encompassing marine biological group.

This department deals mainly with the ecology of bacteria, fungi, and viruses and their role in the turnover of natural and anthropogenic molecules. Because of the Institute's site, much of the research concerns organisms that live in brackish waters. There is also some interest in fish pathology. Specific projects deal with comparisons of stratified microbial communities, deep sea microbiological activity, and the microbial loop.

The departmental faculty is also involved in several cooperative projects, some of which are international in scope. These include studies of the sediment microbiology between Greenland and Norway (Voring Plateau), biological monitoring of the Baltic Sea as required by the Helsinki Commission, the Joint Global Ocean Flux Study (JGOFS), and a program to measure pollutant sources and sinks in the Kiel Bight and the Baltic Sea.

Specific Projects

In the Marine Microbiology Department, several laboratories are worthy of in-depth discussion.

Professor L.A. Meyer-Reil's Laboratory. Much of the work of this laboratory concerns the metabolic activities of marine bacteria, especially those in deep-sea sediments. Professor Meyer-Reil is an exponent of a method that depends on the incorporation of ^{14}C-or^{3}H leucine into bacterial protein to measure bacterial growth. I believe it is rather dangerous to assume that leucine incorporation measures growth rather than protein turnover--a process stimulated when bacterial cells are starving. Sometimes this procedure is supported by information from ^{3}H-thymidine incorporation into DNA. Where similar results are found by both methods, it is likely that both molecules are incorporated intact. These techniques have been used with core samples from the Voring Plateau in the Norwegian Sea. Meyer-Reil's group has found that detritus added to core samples stimulates microbial activity. However, as they point out, great care is needed in interpreting the experiments because perturbation of the sediments alone causes increases in activity. They now consider that in these particular sediments, foraminifera are involved to a greater extent than bacteria in early remineralization processes. Work is progressing on equipment to measure bacterial activity remotely in sediments at great depth. At

present, only trials in shallow water on beaches have been attempted.

Dr. H.G. Hoppe's Laboratory. Dr. Hoppe's group is concerned with water column bacteriology, particularly vertical profiles of bacterial activity and biomass. They have worked in the Arabian Sea, on a 20°W transect in the North Atlantic (JGOFS), and, of course, locally. They find their methods in general produce results similar to those of their U.S. colleagues. This laboratory has promoted the use of fluorogenic substrates to measure the activity of extracellular hydrolases. They argue that because much of the organic carbon and nitrogen in the sea is macromolecular or particulate, the limiting step in bacterial production is the rate of solubilization of these resources. Thus, they have measured the release of fluorophores from fluorogenically labelled organic matter. Currently, they are determining peptidase activity as a measure of the release of organic nitrogen. In the North Atlantic, peptidase activity/cell increased slowly with depth down to 1,000m.

Dr. G. Rheinheimer's Laboratory. Dr. Rheinheimer is the Director of the Marine Microbiology group in Kiel. His laboratory is currently working on the degradation of pollutants in the Elbe estuary. Curiously, Dr. Rheinheimer and colleagues found that a model pollutant such as p-nitrophenol is degraded much more slowly by marine than estuarine populations of bacteria. The reasons, Dr. Rheinheimer believes, are related to the prehistory of the cells, because acclimation of the marine population to p-nitrophenol produced degradation rates comparable to the estuarine samples. This implies also that levels of p-nitrophenol pollution in the estuary were higher than in the open ocean. A potential further complication in measuring environmental rates of bacterial degradation of anthropogenic materials is the presence of grazing protozoa which effectively reduces the numbers of active bacteria.

Other Programs

Other efforts in the department are devoted to microflagellate ecology, nitrogen dynamics in sediments, ciliate grazing, fish pathology, and the endosymbionts of bivalves.

Dr. W. Reichardts' Laboratory. As can be expected in an institute of this size (especially one where there are departments dealing separately with the phytobenthos and the plankton), other microbiological investigations take place outside the Department of Microbiology. Dr. W. Reichardts' laboratory is an active example of this phenomenon. His main thrust concerns the interaction of bacteria and invertebrates in anoxic marine sediments. However, much of the work is descriptive, and little is known concerning any physiological roles for these epizoic bacteria.

Concluding Remarks

The Department of Microbiology at the Kiel Institut für Meereskunde deals largely with descriptive microbial ecology. No one I met could be regarded as working at the molecular level. Perhaps a conscious decision was made to leave more molecular studies to their colleagues at the nearby limnological institute at Plön.

France

The Station Biologique de Roscoff

Introduction

The Station Biologique de Roscoff (the Station) is affiliated with the Centre National de la Recherche Scientifique (CNRS) and the Université Pierre et Marie Curie in Paris. Roscoff, once a pirate stronghold, is a small town in Brittany and is most conveniently reached by sea via ferry from Plymouth, United Kingdom (U.K.) or by air via Brest. The town flourished in the late middle ages but has existed since the Roman occupation of Gaul. Much of the present town dates from the 16 or 17th century, making it a delightful place to visit. For those not familiar with this part of Europe, I should emphasize that Brittany is the Celtic region of France--an area with its own historical language and traditions. As in other Celtic areas of Europe; e.g., Wales, there is a growing interest in preserving their ethnic heritage.

The marine station was founded in 1872, 5 years before the Marine Biological Association Laboratory at Plymouth, U.K. The library of the original station (17th century) is still used today, but that is the only old-fashioned aspect of the work at this institute. The director, Professor Pierre Lasserre, explained that the focus of his institute was marine biological rather than biological oceanography. I would add the adjective molecular to marine biology because everyone I interviewed was working at that level. This institution is quite distinct from the nearby (Brest, 30 km) laboratories of L'Institut Francais de Reserche Pour L'Exploitation de La Mer (IFREMER) where the research carried out, as the name suggests, is more applied than that in Roscoff.

The Station has 55 (15,000m^2 total) modern, dry as well as traditional wet, laboratories. There are three boats (8m, 12m, and 20m) capable of working in the shelf environment. Historically, much of the work has been stimulated by the local intertidal environment that, given the approximately 8-m tidal range, is enormous. Thus, this always has been a center for intertidal invertebrate (4,000 species) and macroalgal (700 species) research. One of the early (1880) achievements at the Station was the construction of a vivier or holding tank for seawater and marine life. This extremely large tank (about 30x20x5m) was rebuilt in 1987 and, of course, since the sea retreats almost over the horizon twice daily, it is essential as a source of water for the wet laboratories. Professor Lasserre emphasizes the local environment, however several of the approximately 100 staff are involved in Antarctic research, the Joint Global Oceanographic Flux (JGOFS), and oceanic frontal studies (FRONTAL, ALMOFRONT, see page 11).

Although the Station could be regarded as somewhat off the geographic beaten path in Europe, it is certainly not isolated from the rest of the scientific world. The laboratory has active research collaborations with the Plymouth Marine Laboratory (PML) and the Center for Marine Biotechnology at the University of Maryland, to name but two (there are many others).

Professor Lasserre is the President of the recently formed European Marine Research Stations Network (MARS). I have established the Office of Naval Research European Office (ONR Europe) as a liaison for this organization to the National Association of Marine Laboratories in the U.S. Our aim will be to foster joint scientific research and exchange of personnel. Participation in cruises is a particularly fruitful way of achieving this. To this end and with ONR Europe support, Dr. Daniel Prieur from the Station visited the U.S. in early 1991 to arrange U.S. participation in a cruise to thermal vent areas in 1993. The cruise will use a French submersible and has a microbiological component.

The laboratory is well situated with respect to the support of visitors having accommodations (80 rooms) and access to cooking facilities. At the time of my visit, there were two U.S. students in long-term residence from University of California, Santa Barbara, and the University of Maryland, respectively. Annually, about 200 short-term visitors are accommodated.

The station has recently instigated a series of workshops commemorating the work done there by Jacque Monod. Details can be obtained from Professor Lasserre. With local accommodations and a modern 150-seat auditorium, this workshop series is well supported. The Station is divided into seven research groups, somewhat along the same lines as U.K. government-supported laboratories. I visited six of these groups.

Selected Research Programs

Several research programs are worthy of in-depth discussion.

Dr. Bernard Kloareg's Laboratory. This laboratory began in its present form in 1989 with the arrival of

Dr. Suzanne Loiseaux de Goer. Dr. Kloareg's laboratory receives funds from the national government under a regional (Brittany) industrial development grant (biotechnology) and from two companies. This laboratory is devoted to the study of macroalgae at the molecular level. The research has both fundamental and applied emphasis. The basic research is concerned with the taxonomic evolution of the macroalgal chloroplast and is based on chloroplast DNA analysis (Dr. de Goer). The more applied research concerns macroalgal products and uses research highly appropriate to the local economy where algothérapie has been practiced for almost 100 years. Brittany is the world's second largest producer of carageenans (the U.S. is the largest).

Dr. Kloareg also is interested in the use of macroalgae in modern biotechnology. To this end, he is researching means to produce protoplasts and ways to regenerate whole plants from them after making genetic fusions. His emphasis is on producing novel cell-wall polysaccharides. Much effort has been expended on finding bacterial enzymes (lyases, fucanases) to degrade the macroalgal cell wall in the production of protoplasts. Currently, an efficiency of 50 percent has been obtained (number of protoplasts achieved compared to number of cells treated). Regeneration of these protoplasts can be synchronized. The gene has been cloned specifying at least one bacterial carageenase used in the production of the protoplasts.

Besides their traditional use in food (nori, gelling agents), seaweeds are also used as fertilizer. Seaweed extracts are sprayed on leaves and improve plant growth. Whether this effect is nutritional (and thus can be classified simply as that of a fertilizer response) or is something more obscure, is not known. Dr. Kloareg has funds to investigate whether the extracts contain any elicitors of cellular response (plant or animal). The project is supported partially by a local company and could not be discussed in detail.

His future research will concern studies of other bacterial and macroalgal polysaccharidases using macroalgal polymers as substrates (agar and caragenans). He will also clone the bacterial and algal genes for these enzymes.

Dr. Kloareg's group contains about 10-12 people at a time, depending on the number of visiting investigators. The ready availability of macroalgal protoplast in this laboratory identifies it as an excellent site for molecular biological research using less studied, but biotechnologically and biologically important, species.

Dr. Laurent Meijer's Laboratory. This laboratory is devoted to the developmental cell biology of *Xenopus* (toad) and *Sphaerechinus* (sea urchin) eggs. His research is typical of that which contributed to the general scientific reputation of many of our modern marine laboratories. By this, I mean that the research is cutting-edge basic biology that uses marine organisms only for convenience. In particular, the research carried out here concerns the regulation of the transition from the G_2 to the M stage in cell division. A cytoplasmic entity known as maturation promoting factor (MPF) is well documented in cell biology and is not species specific. Further, during MPF function, protein phosphorylation reactions occur that may be catalyzed by a histone kinase activity usually known as H_1K. The H_1K activity peaks at the same time as maximal MPF action. Attempts to separate the activities have in fact failed. Currently, Dr. Meyer believes that H_1K and MPF are identical.

Investigations about the controlling factors of cell multiplication have obvious implications for cancer research. The French Cancer Research Association supports this work. Dr. Meijer collaborates with workers in the U.S. and throughout Europe. His laboratory is a European Center for sea urchin cell biology and has published a user guide for the uninitiated.[1]

Dr. Daniel Prieur's Laboratory. Dr. Daniel Prieur operates the only focused marine bacteriological group at the Station, although several other scientists are involved in microbiological work. Before he came to the Station, he was at the IFREMER in Brest. Like many of his colleagues at the Station, Dr. Prieur has several on-going collaborations throughout Europe and the U.S.

There are about 12 people in this deep-sea microbiology group at present; half are students. All aspects of the functioning of organisms at depth are being investigated. The emphasis is in the physiological ecology and potential biotechnological exploitation of baro-thermophiles.

Early general work was on hydrothermal vent microbiology--N-cycle, methane oxidation, sulfur oxidation, sulfate reduction, nonspecific heterotrophs, and heavy metal resistance. He has now added more focused work on bacterial exopolysaccharides that bind heavy metals, ultra-thermophile physiology and molecular taxonomy; e.g., 16S RNA and phospholipid methylester studies, on these organisms. Already it appears that some vent microbes do not have a typical procaryotic lipid profile. A further, but minor, topic (one student) involves bacterially enhanced corrosion and the part that microorganisms play in controlling O_2-fluxes at corroding surfaces. This topic has Italian collaborators from the Marine Corrosion Institute in Genoa and appears similar in emphasis to a project at the Department of General and Marine Microbiology in Gothenburg, Sweden. (Drs. Malte Hermansson and Amelie Pedersen). Dr. Prieur is involved in the international RIDGE project to understand the geodynamics of oceanic crustal accretion. Prieur's

[1] Cahlers de Biologie Marine. 1984. 25:457-480

microbiological focus will be part of the French-American Ridge Atlantic Program (FARA).

Dr. Serge Poulet's Laboratory. The plankton and primary production group led by Dr. Serge Poulet has about 12-15 people, depending on the number of guest workers in the laboratories. These efforts center around four themes: (1) chemistry of the pelagic ecosystem, (2) biochemistry of plankton production, (3) the microphytobenthos, and (4) trophic relations of the zooplankton. The laboratory lists many collaborators in various marine laboratories world wide.

Much of the plankton group's international collaboration occurs through their study of oceanic fronts. Fronts are boundaries between offshore and inshore water masses. Among other things, their study involves interchange between the water masses. In areas where fronts occur, enhancements of biological activity are believed to take place, particularly primary productivity and all that it entails. There is interest in determining how significant these areas are in terms of global productivity and their contribution to biogeochemical carbon flux. As always, mathematical simulations based on such things as satellite imagery, require *in situ* information to validate the models. The group of French workers organized under the auspices of the FRONTAL program have joined an international group to consider the Almeria-Oran (Spain-Algeria) front in a program named ALMOFRONT. As the name suggests, this front, which consists of modified Atlantic water and Mediterranean surface water, runs from Spain to the North African coast. Dr. Poulet, as well as Drs. A. Sournia and P. Morin, are involved in ALMOFRONT. Dr. Poulet's interest is in the relationship of vitamin C in copepods to the general availability of food (measured as particulate chlorophyll concentration). He believes that it is important to monitor vitamin C in food chain work and that this parameter would be useful in a chemical description of frontal dynamics. Dr. Morin's interest is in nitrogen turnover in the frontal area. Dr. Sournia is interested in learning whether the high concentration of phytoplankton in fronts is because of accumulation or growth. These are rather basic questions considering the expenditure of effort on plankton food chains in such oceanic features. His group will measure chlorophyll synthesis rates.

A related interest of this group is the involvement of chemoreception in feeding response of zooplankton. This, Dr. Poulet pointed out, is part of an European Community (EC) project in interspecific communication and relates to the physiological basis of patchiness. This project is funded under the auspices of the Transnational Programs effort, not the Marine Science and Technology Program. Considerable progress has been made in learning about the responses of copepods to allelochemicals. Potential allelochemicals in seawater have been defined by using high-performance liquid chromatography. As a result, dissolved free amino acids and ascorbic acid have been selected for testing in an assay that depends on feeding appendage movement in zooplankton. The rate of movement of feeding appendages, which is a measure of feeding effort, is measured. They use a method that depends on impedance measurements; i.e., the apparatus works on a principle similar to that of a Coulter Counter. A shift in the vibration rate from 20 to 26Hz indicates that the compound being tested is sensed by copecods. Compounds found effective so far are leucine, ornithine, and lysine. Although this project is essentially a basic marine biological study, the approach has considerable promise as a test system for detecting behavioral modifications caused by sublethal pollutant concentrations.

Other interests of this group include toxic bloom algae, the use of flcw cytometry in defining marine planktonic populations (Dr. Vaulot), and a project on the relationship between the abundance of antarctic krill and copepods and dissolved free amino acids. The latter research is a collaboration with the British Antarctic Survey (Cambridge) and the PML, England.

Professor Pierre Lasserre's Laboratory. The group concerned with ecophysiologic and benthic fluxes is lead by Professors P. Lasserre and A. Toulmond. Because of time limitations, I spent only a very short period with this group. Their research is organized around four themes: (1) respiratory exchange in marine animals, (2) regulation of nitrogen excretion, (3) use of microcalorimetry to measure energetic fluxes at the benthic interface, and (4) nutrient dynamics in general at this interface.

I did not visit the group organized under the heading of "Biology of Marine Species." These researchers seemed to be less well integrated into the overall organization than those in other groups.

Concluding Remarks

I can recommend this research station as a place for a sabbatical leave or with which to arrange collaborative studies. The staff is enthusiastic and the laboratories are well equipped. Everyone I spoke with had one or more active collaborative projects already within France, within Europe, or, quite often, with American investigators.

the Netherlands

The Laboratory for Materia Technica, State University of Groningen

Introduction

The Laboratory for Materia Technica is part of the Dental School of the University of Groningen, the Netherlands. This year it is celebrating the 20th anniversary of its formation. The biological adhesion group (10 people) is quite large for such a well-defined subject. However, as is known to people in this field, information arising from particular, fundamental studies of the adhesion and colonization process can be applied generally in other fields, especially in marine science. Dr. H. Busscher told me that the group has a 5-year budget cycle and that salaries are included in the intramural budget allocation.

Flow Cell Analysis of Adhesion

The forté of the group is computer analysis of images obtained from microscopic studies of cellular colonization of physico-chemically characterized surfaces. Both bacteria and fibroblasts are studied. The heart of these studies is a flow cell manufactured from nickel-plated brass. I assumed the construction materials were chosen for ease of machining rather than their inert properties. Some cell lines do not thrive in this flow chamber. This may be a reaction to the nickel coat. The flow cell is equipped with upper and lower transparent windows (approximately 13x5 cm) sealed with O-rings. The windows can be replaced by other substrata but, obviously, one must remain transparent. The main procedural advantages to the cell are that it can be disassembled for cleaning, it allows both laminar and turbulent flow, and the gap between the upper and lower transparent surfaces can be varied using Teflon® spacers. The transparent windows can be characterized by whatever technique considered necessary, before and after an experiment. For instance, where the windows are made of glass, contact angle measurements showing complete wetting of the surface indicate that the glass is free from hydrophobic contamination before an experiment is begun. Such measurements are facilitated in this laboratory since they are computer aided. A video image of the contact angle that the drop of analytical fluid makes with the surface to be tested is used in a curve-fitting program. Thus, the liquid-surface contact angle measurement is generated by the computer rather than by eye using a goniometer. The system can be calibrated using a steel ball in place of the analytical fluid.

Colonization Patterns

Dr. Busscher is a strong exponent of the use in microbial attachment studies of flow cells and continuous image analysis over quiescent systems, point sample times, and manual cell counting. However, he has said in print that he has discovered no principles using his system that were not known before from studies where hydrodynamic parameters were less well controlled. As Busscher points out, where the adhesive interaction of the cell with the surface is low; e.g., many types of cells on hydrophobic surfaces, gross artifacts in counting adhered cells can occur. These are caused by rinsing or draining of the colonized surface. In such cases, surface colonization patterns (a subject that is a main thrust of this group) cannot be observed. Busscher and his group believe that bacterial cells are not randomly distributed over a surface undergoing initial colonization. I believe that unless a patchiness in distribution is visually obvious, it is hard to prove (even with a computer). As the colonization proceeds, there is less room for each incoming cell on a surface. Therefore, it is more likely to attach to a site near to another cell. Thus, a nearest-neighbor analysis of the process may suggest cooperativity where none is actually present. However, work from this laboratory has shown that where a surface is subject to flowing liquid, depositing cells are subject to the micro-hydrodynamic effects of cells already attached, even where the gross flow characteristics are laminar. The onset of macroturbulent flow in the flow cell can be seen since cells not firmly attached to the substratum begin to move laterally about their attachment point; i.e., they wobble.

Steps in Colonization of Surfaces

Studies have shown that with some organisms, colonization is a two-step process. In the first step, often taking 2 to 3 hours, organisms attaching to the surface follow a saturation curve. After a plateau of about 1 hour, further increase (step 2) in cell number takes place. This

is accompanied by secretion of a surfactant by the organisms. In my opinion, this phenomenon is growth related and not necessarily a specific function of metabolism during the colonization process. Such observations of the kinetics of adhesion are much easier to make in an apparatus that allows real-time measurements.

Influence of Adsorbed Materials on Adhesion

In a collaboration with the Center for Biomedical Technology and the Department of Cell Biology at Groningen, Busscher's group is investigating a paradox central to the question concerning the means by which cells of all types interact with substrata of differing surface chemistry. Why do cells adhere differently, both in kinetics and extent, to substrata of differing surface energy when we believe they are separated from that surface by a layer called the initial conditioning film? This film is adsorbed to a surface more quickly than living cells. Dr. Schakenraad, who works closely with Busscher, referred to the ability of the substratum to "shine through" the adsorbed layer and thus influence the cells. Using adsorbed proteins (fibronectin, albumin) as model conditioning films, these workers conclude that surfaces of differing surface energies cause the protein to adsorb in distinctly different island-like patterns, as seen after fixation and examination in the transmission electron microscope. Therefore, in their opinion, the "shining-through" phenomenon is explained partially by the patchiness of the adsorption of the conditioning film, thus leaving some of the substratum uncovered, and also by the effect of the substratum on the three-dimensional structure of the adsorbed material ; i.e., the answer is not black or white but shades of gray.

Production of Polymers with Novel Surface Characteristics

Some of the research is directed towards designing the ideal human implant material. Thus, with colleagues in the Center for Biomedical Technology, they have been able to modify the surface of the fluoroplastic Teflon® while retaining the bulk properties of the plastic. As an example of why this is necessary, Drs. Schakenraad and van der Mei explained that gut patches used to repair abdominal trauma required the patching material to integrate with the tissue in the wound but not with the intestinal tissue. The same dual function is required of vascular prostheses.

FEP-Teflon® is normally highly hydrophobic with a water contact angle of about 109°F. However, after treatment with an argon ion beam in high vacuum using a modified sputter coater, both hydrophilic (water contact angle 6°) and super-hydrophilic (water contact angle >140°) surfaces could be obtained, depending on the conditions used. Whereas the surface of unmodified Teflon® is generally smooth except for surface imperfections, the modified material as seen in the scanning electron microscope (SEM) has a brush-like surface. In spite of the gross change in appearance in the SEM, the measured surface roughness parameter did not change appreciably (from 0.4 μm to 0.5 μm). Examination of the surface by x-ray photon spectroscopy revealed that the carbon-to-fluorine and carbon-to-oxygen ratios were different from the bulk material. Although some fibroblasts were able to spread on the untreated Teflon®, the majority of cells did not. On the hydrophilic material, many cells spread; on the super-hydrophobic material, none did.

The modified Teflon® has been used successfully in a rabbit carotid artery replacement, showing that indeed the ion bombardment creates surfaces that are useable. The procedure has been patented in the Netherlands and the discoverers have embarked on a search for licensees. It should be noted that workers from the Industry-University Center for Biosurfaces at Buffalo (which is supported by the Office of Naval Research (ONR) Ocean Biology/Optics/Chemistry Division) have made similar observations and have been awarded a U.S. patent. Seemingly, these new surfaces and the chemistry behind them may be useful in defining the directions for the chemical syntheses of fouling release surface coatings.

The flow chamber mentioned earlier has been used to measure the hydrodynamic forces necessary to remove bacterial and fibroblast cells from surfaces of differing surface energy. Cells are attached under conditions where there is no flow, and then a flow is started. The video frame-grabber stores the image as the flow is increased stepwise every 15 min in 3 ml min^{-1} steps. Although removal is easy to detect with bacteria, fibroblasts change the degree to which they are spread as a function of shear; i.e., they exhibit a hydrodynamic stress response. The degree of spreading can be measured since the image analyzer can calculate area/cell. The results are recorded as the time to remove half of the initial population of cells ($T_{1/2}$). $T_{1/2}$ values for the following surfaces are FEP (Teflon®) < polymethyl-methacrylate = glass < tissue culture polystyrene: 4 < 72 = 85 < 195 min. Shear values up to 400 dynes cm^{-2} can be generated in this equipment. This approach has obvious utility in assessing the performance of nontoxic antifouling surfaces.

Are the Adhesive Properties of Cells Taxonomically Relevant?

In taxonomy, organisms with similar names are supposed to be related genetically. Bacterial species often bear names related to their ecological niche, which in turn is related to their physiology. Only recently has the adhesion of cells to surfaces become an interest of the microbiologist. Yet, whether a cell has this property is of considerable importance in determining, for instance, its pathogenicity or other aspects of its ecology. Adhesion to surfaces is a multicomponent phenomenon that is not completely understood for any bacterial species. A project directed by Dr. Henny van der Mei has two aims: (1) examining the many cell surface physico-chemical attributes of a series of bacterial strains that may relate to their ability to adhere; (2) comparing these with their traditional taxonomical grouping. Seventeen surface parameters were chosen, including surface charge, hydrophobicity, possession of surface appendages, elemental composition of the surface layers of the cell (by x-ray photon spectroscopy), and infrared spectra of isolates. So far, no striking correlations have been seen. Statistical analysis of relatedness does show some clustering, however. Streptococcal strains form one cluster and staphylococcal strains form another. One of the greatest problems faced by this study is that all of the methods used that measure characteristics of the cells are related to their physiological state; i.e., the measurements are growth-cycle dependent.

Dental Diseases

Although many of the research projects being carried out in this laboratory were at the fundamental level, at least two involved day-to-day dental problems. With dental cavities no longer a serious problem in the Western world, attention is now being focused on other dental diseases. Recolonization of cleaned teeth by bacteria follows an exponential curve, as it does in heat-exchanger tubes in ship's boilers. However, plaque formation slows considerably at night--a situation that does not occur in heat-exchanger tubes! A further component of this study indicates that when one changes the tooth for an intraoral experimental substratum, surface roughness plays an important role in increasing the recolonization rate of cleaned surfaces. It was suggested that the role of roughness may even be greater than that of surface-free energy. I think it is important here to realize that in these experiments, the variable of surface roughness was increased from 0.1 μm to 2.2 μm and this provoked a four-fold increase in bacterial colonization rates. These changes are in the critical range of bacterial size; i.e., c.1 μm. Thus, the situation was changed from one where bacteria were subject to hydrodynamic forces at the surface to one where they were relatively insulated from these stresses. A further increase in surface roughness to, say 10 μm, would not be expected to increase recolonization rates. The plaque-forming species collected by intraoral experimental substrata of differing surface-free energies were not similar, indicating a selective pressure based on the physical chemistry of the surface.

Not all the work of the laboratory uses the flow cell, although at least four of these units, together with the analytical systems, were available. Thus, Dr. Cowan, a postdoctoral fellow from the U.S. (in collaboration with the Catholic University of Louvain) is investigating organisms from the human periodontium. Using a variety of methods, they have found that fimbriate organisms behave differently from capsulated ones and that these differences are evident even within the *same culture*. Fimbriate cells were positively charged and had high nitrogen/carbon ratios in their surface structures. Negatively charged cells were encapsulated and showed high oxygen/carbon ratios within surface structures. Growth in liquid medium favored the negatively charged cells.

Concluding Remarks

The interfacial microbiology group at the University of Groningen Dental School is vigorous with excellent facilities and important collaborative links to other schools in the Low Countries. They are expected to turn their attention to further biofouling projects soon.

Sweden

The University of Gothenburg

Introduction

The coastline of Sweden is roughly equivalent to the combined lengths of those of the Federal Republic of Germany (FRG), France, and the Low Countries. However, the population of Sweden is only a few percent of the populations of those countries. Further, the marine environment varies widely from the Gulf of Bothnia in the north (brackish) to the Skagerrak in the east (North Sea water). Thus, it is easy to see why Swedish marine science has been spread thinly in previous years. Understanding this, in 1989 the Swedish parliament decided to establish three centers for marine science research. These centers were distributed rather evenly around the Swedish coast with two on the east coast (Universities of Umeå and Stockholm) and one in the west at Gothenburg. The role of the three centers is to carry out marine scientific research, monitor the health of the marine environment in their area, educate students at all levels, and assist government environmental agencies; i.e., a very broad task.

I visited the Gothenburg center which is responsible for the coastline from the Norwegian border to the southern end of Oresund. Professor Staffan Kjelleberg, Department of General and Marine Microbiology at the University of Gothenburg, provided the general information in this report. Specific information came from the faculty of that department.

The Marine Research Center of Gothenburg has the most diverse ecological responsibility of the three centers. The responsibility covers the Skagerrak to the southern end of the Baltic Sea--an area where in 1988 an unusual toxic bloom of *Chrysochromulina polylepsis* occurred. This center is governed by a board that oversees the marine activities in the University of Gothenburg (12 departments), the University of Lund, three field stations, and Chalmers Technical University in Gothenburg.

Because marine science is a cross-disciplinary subject, a major role of this center is to stimulate collaborative projects. This is particularly important in a country where the marine scientific population is very small. There has been a concerted effort to fill gaps in technical expertise in this center. The new posts have been distributed between the universities of Gothenburg and Lund. Since this center is less than 2 years old, it has not fulfilled its role as the major surveillance organization for the local marine environment. Ultimately, the three centers will be responsible for coordinating all marine monitoring carried out along the Swedish coasts. I see this as an efficient use of academic scientific talent in a country with a relatively small science base.

The Department of General and Marine Microbiology

The Department of General and Marine Microbiology is one of 12 departments carrying out marine research in the University of Gothenburg and is part of the Botanical Institute (as are the Departments of Marine Botany and Plant Physiology). The types of research carried out are remarkably similar to those supported by the microbiological programs at the Office of Naval Research (ONR), Biological Sciences Division and Ocean Biology/Optics/Chemistry Division. For instance, there are projects that deal with the molecular aspects of biofouling, invertebrate-bacterial interactions, secondary production and the microbial loop, aggregate and microzone microbiology, adhesive synthesis, elemental fluxes (especially N), and biocorrosion. Other areas include differentiation in nonspore-forming bacteria, aquaculture, osmoregulation, and probiotic aspects of fish farming. I will describe some of these programs later in more detail. It should be remembered that this department supports an unusual level of collaboration. Thus, projects I have assigned (in this summary) to one or more persons enjoy scientific input from many other scientists.

Bacterial Adaptation to Stress. Led by Staffan Kjelleberg, the group has two foci. They are (1) adaptation to stress of copiotrophic marine bacteria and (2) interactions between surface-attached bacteria and marine invertebrate larvae. Dr. Kjelleberg points out that much of the work on stringent response brought about by starvation of bacteria has been performed on organisms of limited ecological relevance, especially so as far as the marine environment is concerned. Thus, this group has worked with two marine bacterial species; i.e., (1) *Vibrio* sp.14 and (2) *Pseudomonas* sp. 89. Both organisms are in the culture collection of the University of Gothenburg. Kjelleberg and his coworkers have been able to show that the adaptations to starvation of *Vibrio* has many parallels with sporulation in organisms such as *Bacillus*. For example, miniaturization of cells of *Vibrio*

caused by starvation also induces resistance to heat shock and near U.V. radiation, as well as termination of DNA replication and an increased expression of exoproteases. However, whereas spores of *Bacillus* are dormant, miniature cells of *Vibrio* are involved in low levels (5 percent) of RNA and protein synthesis. These cells do not need an environmental stimulus to "germinate"--only a source of nutrient is required. In spite of the low level of protein synthesis (5 percent of normal rate), the cells contain 25 percent of their usual complement of ribosomes. Quite obviously, these types of adaptation (which involve no commitment step as in sporulation) allow a cell to be a highly opportunistic competitor in an environment whose nutrient concentrations are ever changing.

It has been widely held that cells that have adapted in this manner are in effect dormant, but this may not be true. Recent work by this group has shown that as a consequence of starvation, such nongrowing cells induce a very high affinity glucose uptake system ($K_{1/2} = 500nM$), as well as changing chemotactic receptors. Thus with such a system and a very low metabolic rate, miniaturized cells are involved in the cycling of dissolved organic matter in the sea. Work is in hand to clone the genes responsible for the sequential induction of starvation specific proteins, as well as work on the regulons and signal molecules involved in their expression.

Invertebrate/Bacterial Interaction. This project involves marine bacteria and the larvae of *Ciona intestinales*, *Balanus amphitrite*, and *Ostrea edulis*. Bacterial strains have been found that enhance or prevent larval attachment and metamorphosis. Roles for bacterial polysaccharides are being studied using mutants as well as wild-type strains. At least one low molecular weight compound has been isolated that inhibits both barnacle and ascidian settling. If the molecule is stable and is relatively easily synthesized, it may be of commercial use. If these conditions are fulfilled, Dr. Kjelleberg likely will be approached by an antifouling paint manufacturer to exploit this finding. Again, the approach to be used in future work will involve cloning the genes responsible for the synthesis of the settlement inhibitor. Compounds that stimulate settling are useable in oyster aquaculture. About 12 people are involved in these projects, including one from the U.S. and two from the FRG.

Biocorrosion. Drs. Malte Hermansson and Amelie Pedersen are studying the involvement of microorganisms in corrosion of ferromagnetic surfaces in seawater. (Dr. Pedersen successfully defended her Ph.D. thesis while I was there.) Four strains of marine bacteria were involved in the study which was cosponsored by the Swedish Corrosion Institute in Stockholm (Dr. Vladimir Kuçera). Note that none of these organisms is usually studied in this regard. Early in the project, a novel and inexpensive screening method was developed for corrosive bacterial isolates. The method depends on the solubilization by bacteria of a very thin layer of iron sputter-coated on a glass surface. Solubilization, which is visually apparent, occurs only where bacteria are in contact with the iron surface. Bacterial colonies that cause corrosion can be detected in less than 1 hour. Therefore, the method has obvious use in screening natural isolates as well as mutants with lesions in the pathway responsible for metal dissolution. However, it has not been used for mutant screening. Although this study was started with a view to understanding the microbially mediated process of bio-corrosion, most of the results obtained with the organisms mentioned show corrosion protection. Thus, weight loss of carbon steel coupons was less when in contact with a dense suspension of living bacteria than in a similar suspension of dead bacteria. Apparently, bacterial cells on a surface act as oxygen scavengers and thus remove an important reactant in the corrosion process. Similar results were obtained when corrosion was measured by electrochemical means rather than by coupon weight loss. Where a film of anaerobic bacteria was coated onto the coupon, immersion in a suspension of metabolically active aerobes enhanced corrosion rather than inhibited it. This balance between the two microbially driven and opposite processes may have led earlier to much of the confusion surrounding the involvement of microbes in corrosion.

Dr. Hermansson is also involved in other studies using microbial cells at the marine surface. Like J.H. Paul at the University of South Florida, St. Petersburg, Dr. Hermansson is investigating whether there is enhanced gene transfer between cells on a surface. Certainly, cellular concentration is higher there than in the water column, a situation that should promote the processes of transformation and transduction. Part of the justification for this work relates to the need to understand the fate of genetically engineered organisms released into the environment. Dr. Hermansson also has a project similar to that of D. Kirchmann at University of Delaware in which nutrient scavenging at surfaces is measured. Large (proteins) and small molecules (fatty acids) are used as nutrients. This group has found that the degradation of surface-adsorbed proteins depends on the chemistry of the substratum. To investigate this phenomenon further, they have been collaborating with Dr. Hans Elwing, Department of Physics and Measurements, Linköping Institute of Technology, Sweden, in producing glass slides with gradients of surface energy.

Fish Pathology. Dr. Patricia Conway directs a quite different microbiological project. Her group is involved in studying the bacterial pathology of the gastro-intestinal tract of man, fish, rats, and pigs. They emphasize understanding how changes in surface-bound populations are influenced by nutrient availability. They

have characterized a proteinaceous adhesion from *Lactobacillus fermentum* which is involved in host-specific attachment to a rodent stomach tissue cell line. The aim is to identify the receptor on the host cell. This research has been translated into a related marine project.

Dr. Conway and her group propose to use bacteria isolated from the healthy gut flora of turbot and characterized by their ability to adhere to intestinal epithelial cells, to antagonize gut colonization by the fish pathogen *Vibrio anguillarum*. If successful, these investigators will have developed a probiotic approach to counteract fish disease which is common in fish stressed by maricultural endeavors. About ten graduates are involved in these projects, including four senior staff and guest workers.

Marine Yeasts. A second major set of projects in the department can be categorized also as investigations concerning molecular responses to stress. In these, the molecular basis of salt tolerance in marine yeasts is being studied. Drs. Lena Gustafson and Lennart Adler direct this work, which involves the marine yeast *Debaryomyces hansenii* and *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is being used as an organism for comparison with the marine species and as a help in developing genetic techniques for the less-studied marine yeast. Both yeasts osmoregulate using glycerol, so they are biochemically similar in this regard. Attempts are being made to identify osmotically induced proteins (stress proteins?). Until now, they have found 10 proteins whose concentration is increased by osmotic stress. Both microcalorimetry and ^{13}C-NMR are being used as tools in investigating pathways influenced by a high salt environment.

In discussions concerning the cellular trigger for an osmo-stress response, it was suggested that changes in membrane conformation in relation to turgor may be involved. Currently, there are no well-accepted hypotheses. The team is involved in producing mutants to investigate the means of osmotic stress signal transduction. It is known that it is not high Na^+ concentration alone that prompts the response, but these workers consider K^+ is a possible candidate for transducing the osmotic signal involved in glycerol accumulation.

Marine yeasts, especially *Rhodotorula*, have also been proposed as a food source for salmon mariculture. However, there are problems here. For example in one series of feeding trials, the salmon gut flora was replaced by two wild yeasts unrelated to the food yeast. There are about 14 people working on marine yeasts, including 4 senior investigators.

Groundwater Microbiology. I visited with investigators who were working on two other projects. Neither is marine, but they exemplify the wide range of interests in this department. Dr. Karsten Pedersen has succeeded in growing *Gallionella ferruginea* to 2×10^6 cells ml^{-1} autotrophically under microaerophilic conditions. The characteristic stalk (often about 30 μm long) was not formed during logarithmic growth or at low oxygen tension. In other words, the main morphological characteristic of the cells is not one related to healthy growth, but to oxygen or nutritional stress. Therefore, it is no wonder that for many years it has been impossible to grow organisms that look like the organisms found among the yellow ochre in ditches and wellheads in areas where the groundwater is high in iron.

Dr. Pedersen has other interests which also relate to groundwater movement and microbial growth deep beneath the earth. He is investigating the microbiology in rock where high-level nuclear waste will be stored in copper canisters. It is known now that many organisms growing under starvation conditions produce extracellular, metal-chelating polysaccharides and that these polymers have been implicated in corrosion. Furthermore, seemingly insoluble nuclide glasses may also be made soluble by such chelation effects and leak into the groundwater. Much of the research carried out by Dr. Pedersen has been in 1,000 m bore holes in Swedish granite.

Nitrogen Fluxes in the Sea. I was unable to visit the group working on N-turnover in the sea. However, I found that areas of study include the Skagerrak, Mediterranean Sea, and the Southern Ocean. Both water column and benthic/water column interactions are studied. The group's five staff members collaborate extensively with other marine institutions in FRG, Belgium, and France, as well as field stations in Sweden. The group appears to use standard techniques (^{15}N uptake) to measure nitrogen dynamics. One of their projects is related to the discharge of nitrogenous nutrients to the Gothenburg archipelago from the Göta River, land runoff, and the Gothenburg wastewater treatment plant.

From the above project list, it can be expected that this department runs many two-dimensional gel electrophoretic separations of proteins, as well as DNA sequencing gels. Because of this and the need for complete standardization of such procedures, the department will become the Swedish center for this type of work. They have standardized their methods using the Millipore system and will interpret scanner readouts using a computer. They will provide a service to other investigators in Sweden.

Concluding Remarks

The Department of General and Marine Microbiology is an extremely vigorous department with collaborations extending to many parts of the world. However, they do not rest on their laurels. They realize they are in an excellent position to promote marine biotechnology as an industry for Sweden. Dr. Karin Malmkrona has been appointed as coordinator for the venture.

I spoke at a symposium to launch the idea in western Sweden ("Marin Bioteknik i Västsverige"). I explained the ONR approach to marine biotechnology and the National Science Foundation (NSF) model for industry-university interaction along focused disciplinary lines. Others spoke of contributions that university science could make to the marine industry. Dr. Jan Johannessen, President of the Bergen High Technology Center, Norway, described its formation and future plans as an example of what can be accomplished.

The steering committee for the western Sweden venture was excited by the prospect of promoting marine biotechnology. Consequently, they proposed to join with Dr. Johannessen and agreed also to invite a Danish contingent to join with them to form the Nordic Marine Biotechnology Consortium. Given the vigor of the group in Gothenburg and the enthusiasm from Norway, I expect this endeavor to be successful.

Dr. Johannessen described his new 250,000-sq. ft. building on the waterfront in Bergen. So far, over 500 million NKr (about $81 million) has been raised for the Bergen center with 90 percent from industry and 10 percent from the government. Information technology and biotechnology will be the center's major foci.

Switzerland

The Swiss Federal Institute for Water Resources and Water Pollution Control, Dübendorf

Introduction

The Swiss Federal Institute for Water Resources and Water Pollution Control (Institute), Dübendorf, teaches and carries out research, and is associated with the Swiss Federal Institutes of Technology, more often called by its Swiss acronym, ETH (Eidgenosische Technische Hochschule). In spite of its concentration on research related to public and environmental health, the Institute has no regulatory function. However, it does advise the various levels of Swiss government and industry. Of 200 professionals, postdoctoral students usually form 15 percent, a high level. The Institute offers undergraduate classes in all aspects of basic aquatic science, including oceanography, as well as applied courses in such subjects as wastewater management and sewage treatment.

Continuing education also forms a part of the educational responsibility of this institute. A program that I have not seen for 20 years is one designed specifically for laboratory technicians. The program is an paraprofessional apprenticeship-style course and provides practical experience of all modern chemical laboratory methods.

The research focus of the Institute is at a basic science level, but the projects are related directly to applied problems. The main areas are aquatic ecology, environmental chemistry, process engineering, material fluxes (especially pollutants), and water management methodology. I spent all of my time with those scientists concerned with the aquatic ecology group led by Professor Geoffrey Hamer. Dr. Hamer was one of the first British graduates in the then-new discipline of biochemical engineering. Even 30 years or more later, it is rare to find someone formally qualified both in engineering and microbiology.

I also visited with Drs. Thomas Egli, Tony Mason, and Mario Snozzi. The group is highly integrated and it was unclear which scientist was the initiator of each project discussed. Many publications from the Institute are jointly authored. However, I detected that Hamer's philosophy pervades the projects being carried out.

Professor Hamer's Laboratory. Hamer is refreshingly skeptical of current microbiological dogma about the need for genetically engineered organisms to biodegrade pollutant molecules. He points out their physiological and genetic instability in commercial projects, for instance. Hamer is an advocate of the study of mixed cultures growing on mixed substrates as real-world models. He argues that in nature no degradation is carried out by single species. Therefore, why should we follow that route in commerce? He blames (if that is not too strong a word) the biochemical community for the "obsession" (his word) with single organisms. Of course at first, biochemical investigations needed pure cultures since it was not possible to examine single metabolic pathways without them. Dr. Hamer told me that most mixed cultures capable of degrading rather simple mixtures of organic compounds are usually about 90 percent of one activity (not necessarily one organism, although it might be) and 10 percent satellite organisms that cannot degrade any of the compounds in the initial mixture. These organisms grow on by-products of the feedstock that may inhibit the growth of the majority of the biomass. Dr. Hamer is also critical, with just cause, of the modelers of microbial processes (usually engineers like himself) who insist on using in their material balance equations, data derived from elemental composition of microbes (e.g., particulate carbon) in the absence of physiological information, and biochemical composition of microorganisms. This theme has been developed previously (see *ESNIB* 91-01:5-14).

Dr. Hamer's career has spanned the growth of modern microbial biotechnology. He believes this started with the advent of the single-cell protein (SCP) in the middle 1960s, since this was the first attempt at process engineering based on microbiological findings. I disagree somewhat because there is no doubt that the antibiotic industry, although highly empirical in its approach, did attempt to incorporate O_2 mass transfer information for instance into fermenter design some years earlier. In Copenhagen at the Congress on Biotechnology, I heard Dr. Hamer use this information concerning the SCP industry to warn against developing a biotechnological industry dependent on a feedstock the price of which is regulated politically by the Organization of Petroleum Exporting Countries (OPEC), rather than by its cost of production. When I heard this, I wondered why such an old (25 years) example had been chosen, until it was pointed out that excess agricultural products now fulfill the earlier role of oil. The prices of agricultural products

are completely artificial in most parts of the world. Thus, a biotechnological process can be regarded as economic or not, depending not on supply and demand, but on political considerations. Dr. Hamer expressed particular concern about the situation in the European Community (EC) where a large fraction of the total budget is used to support the common agricultural policy.

In spite of their various skepticisms, the group in Dübendorf is an exciting group to visit. They have advanced towards redressing some of the deficiencies they perceive in microbiological lore. The main projects involve:

- Use of thermotolerant organisms in remediation of petroleum by-products (because it is hot and arid in many hydrocarbon-producing nations).
- Involvement of the cometabolic activities of methylotrophs in bioremediation
- Growth of organisms at very low single and mixed substrate concentrations
- Physiological responses in organisms subjected to environmental transients (O_2, nitrogen sources)
- Microbiological aging and death
- Expression of heat-shock proteins in continuous culture (most previous work has been in batch cultures)
- Bioremediation of solvents and the complexing agent nitro-triacetic acid (NTA).

This is a wide range of subjects, but with the common theme of practical application.

Dr. Thomas Egli and Dr. Mario Snozzi's Group. Dr. Thomas Egli has produced a monograph on the biodegradation of NTA. The monograph reviews the previous work of others and all that produced by this group. Some highlights mentioned by Dr. Egli include the taxonomy of those organisms able to degrade NTA, their distribution, and the fact that they turned out not to be organisms that were known previously. The majority of the organisms isolated, plus two from the American Type Culture Collection, were found to be Gram-negative, obligately aerobic, motile rods which exhibited pleomorphism. This is enough for most biochemists to add these organisms to the enormous list of pseudomonads, which are known to be biochemically diverse in their degradative capabilities. In fact, none of the strains were members of the genus *Pseudomonas*, but some were representatives of a new genus of the Proteobacteria for which the name *Chelatobacter* has been proposed.

A second group were Gram-negative, nonmotile short rods. The name that has been proposed for this group is *Chelatococcus*. Extensive use of 16S RNA techniques was made in arriving at these conclusions. Dr. Egli believes that *Pseudomonas* as a genus may not be as diverse in its metabolic repertoire as is generally believed, because many organisms are included in it on rather inadequate evidence. Of the 11 strains investigated in this study, only one proved to be a known organism and that was a strain of *Rhodococcus*. Using an indirect immunofluorescence antibody test, *Chelatococcus* and *Chelatobacter* were found ubiquitously in the natural environment. Levels tenfold higher were found in sewage treatment plants. Levels of NTA found in the natural waters of treatment plants were sufficient to induce or derepress NTA-catabolic enzymes in cells pre-grown under non-inducing conditions (acetate). Dr. Egli and Snozzi also described the group's work on the utilization of mixed substrates. Usually it is only in the laboratory that organisms are exposed to single growth substrates; in nature and in treatment plants, multiple substrates are the rule. In this regard, industrial effluents, particularly those from petrochemical plants, are considerably simpler than those from the domestic sewage lines. This group is dealing with the use of binary, tertiary, and quaternary substrate mixtures.

One of the major findings of universal microbiological importance is that the phenomenon of diauxie does not occur at environmentally significant substrate levels. In chemostats, glucose is used at the same time as methanol by *Hansenula* (yeast). In batch culture, glucose and galactose are used simultaneously by *Escherichicia coli*. However, diauxie was observed at sugar concentration higher than about 5mg/L, so the text books are partially correct. The work on sugar utilization was facilitated by the development of a sensitive chromatographic analysis for reducing sugars capable of measuring concentrations of $5 \mu g\ L^{-1}$ in a 200 μL sample; i.e., a sensitivity of about lng. The method depends on ion exclusion chromatography for separation of the sugars followed by reaction of the separated sugars with copper bis-phenanthroline and amperometric detection of the reduced copper ion. Samples are electrolytically desalted before analysis. The method has been used on limnological sediment pore-water samples and I see no reason that it could not be used in seawater, since there is an initial desalting step.

The quaternary mixture investigated consisted of the organic solvents methanol, methylene chloride, acetone, and iso-propanol. Iso-propanol was included because of its use in airport deicing treatments. Although methylene chloride in the mixture was degraded completely by the consortial association, none of the organisms in the enrichment could grow on this substance as sole source of carbon and energy.

Dr. Tony Mason's Laboratory. Dr. Tony Mason is interested in cryptic growth and what is needed in the way of heat treatment to kill pathogenic bacteria in sewage sludge. Incineration is not appropriate because of the formation of dioxin, and it also does not reduce the heavy metal content. The group has proposed solubilization and degradation of sludge using aerobic thermophilic bacteria, as opposed to the use of mesophiles under anaerobic conditions. However, this still will not remove heavy metals. At the moment, the experimentation is using baker's yeast and *Klebsiella pneumonia* as a model feedstock, rather than authentic sludge. One of the aims of the process is to minimize the yield of the remediation organisms, otherwise the process merely converts the feedstock into the biomass of the product.

Perhaps it is because Professor Hamer spent time at the Kuwaiti Institute for Scientific Research (biotechnology) that so many of the projects described to me involve work with thermotolerant organisms. As Hamer points out, recovery of process water is desirable anywhere, but it is doubly important in desert areas where it must be regarded as a potential resource. In such areas much of the world's petroleum is extracted, yet most of the technology to treat process waters has been developed in northern climates using mesophilic organisms; i.e., grow below 40°C. Hamer's group has isolated a thermotolerant methylotrophic bacillus able to grow at 57°C on mixtures of short chain alcohols. The bacillus is grown in co-culture with a second bacillus that is not methylotrophic. The behavior of reconstituted co-cultures could not be explained based on knowledge of the individual organisms, indicating organism-organism interaction was taking place.

A final project that I visited concerned the means by which genetically engineered microorganisms (GEMS) could be preserved in the environment. This sounds like a curious project when most people are worried in case they do stay. For a long-term *in situ* biotreatment process using engineered organisms to be successful, the organisms must be long-lived themselves. The project has only just started and no results are available.

Concluding Remarks

The diversity of projects here is enormous. The group is enthusiastic and obviously productive; it is encouraging to see a group of scientists whose backgrounds are so diverse able to complement each other. In talking to them, it is not obvious who are the engineers and who are the microbiologists. This institute would be an excellent choice for a marine microbial ecologist to spend a sabbatical leave. There is ample equipment and the laboratories are well maintained.

United Kingdom

England

Plymouth Marine Laboratory, Plymouth

Although marine biological research has been going on in Plymouth for over 100 years, the entity today known as the Plymouth Marine Laboratory (PML) is new. The PML was founded in April 1988 on recommendation of House of Lords Select Committee on Science and Technology. The Institute of Marine Environmental Research of Natural Environmental Research Council (NERC) and the laboratory of the Plymouth Marine Biological Association were joined to form PML. The PML director recognized that the combined laboratories had three major strengths: (1) the integration of chemical, physical, and biological processes in understanding how the marine ecosystem works; (2) biological productivity; and (3) anthropogenic impacts on the marine environment. Research is still organized in those categories. In each case, effort is concentrated on identifying controlling processes and means to measure their rates. The overall aim of the PML is to understand how to balance exploitation and conservation of the marine environment.

Research in the PML is carried out by informal groups. This is typical of this type of organization in European laboratories. I believe it relates to funding pressures. While most research in the U.S. is carried out by individuals or small teams built by an individual, in Europe research is generated by nationally or internationally focused programs that demand collaboration. This is especially true of European Community (EC) programs, such as the Marine Science and Technology Program (MAST).

In PML there is crossover of personnel between scientific groups. The staff publishes about 120 papers annually, including several by PML's directors. I will summarize work involving some of the 10 research groups.

Biogeochemical Ocean Flux Study

The PML is the host laboratory for the Biogeochemical Ocean Flux Study (BOFS) project. However, there are 15 other United Kingdom (U.K.) research groups involved; e.g., Menai Bridge Laboratories in Wales. The study will define the conditions regulating the rates of oceanic uptake and production of CO_2. These processes control atmospheric CO_2 concentration. This, in turn, is thought to influence global warming. The study was still getting underway when I visited PML. At that time, some effort was directed toward defining the processes involved, rather than measuring their rates.

Dr. M. Wyman is a recent arrival from the well-known cyanobacterial research laboratory of Professor N.G. Carr. This has greatly enhanced the ability of the PML to study the role of the picoplankton in oceanic CO_2 fluxes. It is well known that picophytoplankton (< 1mm) play a major role in the carbon and nitrogen budget of the sea. Although Dr. Wyman came from a laboratory at the exact center of England (and thus far from the ocean!), much of his previous work has been with scientists in U.S. marine laboratories, notably at Santa Barbara, California; Honolulu, Hawaii; and West Boothbay Harbor, Maine.

A recent methodological advance was beneficial to field work with cyanobacteria. Dr. Wyman found that photosynthetic energy transfer in the cyanobacterium *Synechococcus* could be uncoupled with glycerol. This means that *in vivo* fluorescence of *Synechococcus* can be related to its total phycoerythrin content, thus enabling depth profiles related to organism activity to be made.

This technique has allowed work on the response kinetics of eukaryote and cyanobacterial cells in oligothrophic waters to small nitrate pulses. Briefly, the cyanobacteria exhibit small blooms but the eukaryotes do not respond. This work was followed up by chemostat experiments in the laboratory, where it has shown that nitrogen-limited cultures responded to nitrate addition in 1-2 hours. However, phycoerythrin-specific transcripts increase 30 minutes after the nitrate pulse. Using probes, Wyman and colleagues demonstrated that large increases in the size of the RNA pool preceded the shift up in growth rate.

Like laboratories in California, Texas, Maryland, and Bergen, Norway, the PML also has interests in factors causing the collapse of plankton blooms. Drs. Wyman, Joint (from PML), and Carr, University of Warwick, have demonstrated a potential bloom lytic agent in that they have isolated a virus from the open sea that lyses *Synechococcus* WH 7803; i.e., a cyanophage. The phage is marine cyanobacterial specific and does not lyse fresh water cells.

Dr. Wyman summed up his interests in the phytoplankton in one sentence. He said, "What are they doing and how fast do they grow?" I was interested to note that he did not ask how many are there!

Dr. Carol Turley came to PML from the Menai Bridge Laboratories. She is interested in the process whereby carbon is exported to the ocean depths by means of aggregate formation. She has noted that seasonal sedimentation of such aggregates in the North Atlantic also causes the co-transport of substantial amounts of relatively undegraded pico- and nanoplankton to the seabed (4,500m). She believes that it is unlikely that such small single cells would become sedimented alone. The cyanobacterial component of the aggregates is autofluorescent, indicating that these cells are transported rather quickly. Laboratory incubation of abyssal material under *in situ* conditions demonstrated its rapid degradation. Work from other laboratories suggests that these aggregates are not degraded quickly in the deep sea. Dr. Turley collaborates with her former graduate student colleague (University of Wales), Dr. Karin Lochte. Dr. Lochte is now at the Polar Research Institute in Bremerhaven, Federal Republic of Germany (FRG).

Dr. Robert Williams has wide-ranging interests that relate to an overall theme of zooplankton feeding and ecology. Part of the work has been to define the boundaries of assemblages of zooplankton found initially from continuous plankton recorded data. As is widely known, PML has been a pioneer in continuous plankton surveys, especially for the use of recorders towed from ships of opportunity (i.e., commercial vessels plying certain trade routes so surveys can be taken on each voyage). Data acquisition on such a cruise is about 50 percent effective compared to 95 percent on a research cruise. Since the cost of these cruises is effectively zero, this represents extremely cost-efficient data collection.

Probably because of their previous successes in this field, PML has now undertaken designing and manufacturing towed sensor arrays. This project is a cooperative arrangement with Chelsea Instruments Ltd, U.K., and the Institute of Oceanographic Sciences, Wormley, U.K. A current version is *Aqua Shuttle* which is equipped with 11 sensors (conductivity, turbidity, temperature, fluorescence, depth, and 6 light sensors). The sensors are read every 10 seconds. An onboard microprocessor allows the towed vehicle to undulate between preset depths with a particular periodicity. Planned additions to the sensors are for dissolved CO_2, NO_3^-/NO_2^-, and bioluminescence. The British Ministry of Defense (MoD) has expressed interest in the bioluminescence sensor. NO_2^-/NO_3^- sensors can be used to detect fronts. So far, biofouling of sensors has not been a problem, but the longest tow has been only 14 hours. The work is supported by the MoD and Ministry of Agriculture, Food and Fisheries (MAFF). According to Dr. Williams, the Naval Oceanographic and Atmospheric Research Laboratory, Bay St.Louis, Mississippi, has also shown interest in this project.

Dr. Williams also has a joint project with Dr. Serge Poulet which is funded by the EC MAST program. I will describe this project in the section on the Station Biologique de Roscoff, France.

Dr. Roger Harris (group leader for the BOFS project) and Carman Morales (Spanish postdoctoral fellow) are using measurements of plant pigments in the gut of zooplankton and fecal pellets to estimate gut clearing rates of ingested phytoplankton. A 'chlorophyll equivalent feeding rate' is obtained from these measurements which in turn depends on the fluorescence of the pigments. A problem mentioned was the size of the conversion factor from *in situ* chlorophyll fluorescence to carbon ingested. However, the method has been adopted for the BOFS component of the Joint Global Ocean Flux Studies (JGOFS). The method will be used to estimate the fecal component of aggregate sinking from the euphotic zone. Dr. Harris believes that no satisfactory methods exist to measure zooplankton feeding, and none of the methods used so far consider food quality.

Dr. Peter Burkhill is involved in studying the role of protozoal grazing of picoplankton. Because of their small size and thus their lack of grazing by larger zooplankton, it has been suggested that picoplankton form a carbon sink. To measure predator/prey interactions, Dr. Burkhill is using the heterotrophic protozoan *Oxyrrhis marina* as a predator and the chlorophyte *Dunaliella tertiolecta* or similarly sized polystyrene spheres as its prey. Preliminary work using a flow cytometer has shown that the protozoan at first cannot distinguish between real and substitute prey, but after about 20 minutes exposure, they ingested the polystyrene beads less; after 2 hours, not at all. Thus, they appear to learn to distinguish between real and substitute prey.

Dr. Burkhill also proposes to utilize 18S RNA technology to help in the taxonomy of small, 3-5-μm microflagellates. He believes they are a neglected group of organisms in the oceanic microbiological food chain. He hopes to use fluorescently labelled oligonucleotide probes targeted against the 18S-r RNA. Undoubtedly, marine biology can benefit from the technologies developed in molecular biology. Apparently, there are

some basic problems of measurement using existing techniques (see *ESNIB* 91-02:35-36). Another problem mentioned by Dr. Burkhill is that during the recent (1989) cruise at the JGOFS sites (47°N to 60°N on 20°W longitude), American estimates of primary productivity were three times the British estimates. The German and British estimates were similar. So far, there is no explanation. Dr. Burkhill's laboratory has considerable experience in operating flow cytometers at sea.

Physical Process and Growth

Dr. Ian Joint is the project leader for a project that involves physical processes limiting growth of pelagic organisms. This is based in the North and Irish Seas and involves measuring the influence of upwellings and fronts on primary productivity and bloom formation. Dr. Joint told me that at the mouths of some European rivers, a large fraction of the phytoplankton derive their nitrogen from ammonium rather than nitrate. This finding will impact proposed EC legislation to reduce riverine input of nitrate to estuaries.

A recent problem on European beaches is the result of offshore *Phaeocystis* blooms. Although not known to be toxic, the foamy mess thrown up on the beach is considered a nuisance. The popular press regarded these phenomena as pollution related. Archived data (1946-1987) from the continuous plankton record taken in the North Atlantic and the North Sea show that, in fact, there is less *Phaeocystis* in the phytoplankton than in previous years. This fact and recent cruises to measure primary productivity in the Irish Sea point out how little we really know of the workings of the ocean. The PML cruises to the Irish Sea in 1987 and 1988 measured productivities that differed 10 fold. At the moment, there is no explanation for this.

Molecular Studies Group

Dr. Michael Moore is project leader of the molecular studies group. They are investigating the means of communication of environmental information to the DNA of the cell and are trying to understand the causality between stimulus and cellular response. The organisms being studied are either marine invertebrates or fish, and the challenges used are pollutants; i.e., these are ecotoxicology studies. Work is progressing to use tissue cultures in place of whole animals. In particular, this group is working to perfect cell cultures from dab, plaice, and flounder. The work is similar to that being carried out at the University of Stirling.

Specific projects with mussels (*Mytilis edulis*) include enhancement of oxyradical generation in the digestive glands, stimulation of lysosomal activity in pollutant-stressed organisms, immunology of blood cell surfaces using lectins, and molecular genetic analysis of estuarine and coastal populations. Preliminary results show that coastal populations are genetically uniform, whereas estuarine populations are heterogenous. The population analysis is being extended to *Sagitta* (Arrowworms) and deep-sea vent polychetes.

In other research in this group, the immunology of bottom-living flatfish is being studied. Such creatures are particularly susceptible to pollutant stress which leads to immunosuppression and disease.

Marine Biological Laboratories

We visited one project in the Marine Biological Association Laboratory that still maintains a separate visibility in the consortium. Drs. C. Brownlee and J. Green are investigating the influence of low-frequency magnetic fields on the ability of diatoms to move on the surface of agar. This project, a joint one with St. Bartholomews Hospital, London (Dr. R. Dixey), is funded by a power generating company. The research agenda set out by the funding agency includes the experiments necessary to repeat exactly those published earlier by an American group in which our laboratory at Montana State University took part by designing the experimental protocols to be used. However, we did not take part in the data collection. For the new study, our laboratory has supplied cultures of the organism used in the original study (*Amphora coffeaeformis*) and details of our unpublished results. Evidently, results published earlier have generated some controversy. The aim of these workers is to show a calcium flux (or more likely a concentration transient) using a fluorescent calcium-specific fluorphore. A calcium microscope is available in this laboratory. Scientists in this laboratory have published several papers on intracellular calcium concentrations in a diatom and macroalgae spores.

Concluding Remarks

The PML is a modern marine institute whose focus is largely biological and spans the field from molecular to population studies. The newly formed Marine Research Station Network of major EC laboratories will hold its first courses in marine molecular biology here in 1992. Thus, I expect the PML to maintain its leadership in nonmicrobial molecular studies and to move towards the application of molecular techniques at the lower end of the food chain.

The British Antarctic Survey, Cambridge

Introduction

The British have been exploring the Antarctic since Captain James Cook first visited the area in 1772. When he returned in 1775, Cook wrote--"Should anyone possess the resolution and the fortitude to elucidate this point by pushing yet further South than I have done, I shall not envy him the fame of his discovery, but I make bold to declare that the world will derive no benefit from it." The point referred to concerned the existence of Antarctica.

In spite of Cook's remarks, since then Antarctica has attracted sealers, whalers, more explorers (including most recently, Robert Scott and Ernest Shackleton), and nowadays scientists. The British have five bases--all at coastal sites. In 1985, the now infamous ozone hole was discovered at one of them. All British research in the southern oceans and land masses is channeled through the British Antarctic Survey (BAS). The BAS was formed from the Falkland Islands Dependencies Survey in 1967 when it became a NERC Institute.

Macrobiology

Dr. Andrew Clark directs the BAS Marine Life Sciences Division. He told me that there are three scientific groups. However, only the so-called off-shore research group is involved in work at the molecular or microbiological level. The biological research carried out here is mostly macro- rather than microbiological. Much of the marine biology involves fish stocks and their higher predators, areas that are not within my task. However, I found it highly innovative. For instance, bird occupancy of nests and chick growth can be measured by encouraging birds to nest on top of digital balances. By recording baseline weights, the increase by nest occupancy can be detected. The average weight increase of total offspring can be recorded too with all the information being transmitted to a remote station. This approach is certainly a warmer way to gather bird behavioral information than using a pair of field glasses in a blind.

Krill are a major concern of the zooplankton group. They have developed side-scan sonar to follow krill assemblages and can distinguish between fish schools and krill by their acoustic signature. Krill are most reflective at 120 KHz whereas fish are detected best at 38 KHz. However, the acoustic reflectivity of krill depends on their reproductive and nutritional status. For instance, egg masses have a different reflectivity than the krill body. They have found that most krill breeding takes place within the assemblage, but there is sufficient extra-assemblage mating to prevent the formation of races. There has been some work in trying to age krill. What appeared to be a promising general technique did not work for krill. However, it was successful with other invertebrates. The pigment lipofuscin, which is fluorescent, appears to increase with age. In crabs, it is deposited in neural tissue; in bivalves, it is found in the shells.

Microbiology

Dr. D. Wynn-Williams, a microbial ecologist, alerted me to a technique from BAS that had general relevance in microbial enumeration studies. It is well known that staining of bacteria with fluorescent dyes makes them easier to count. One of the problems of this technique, especially when automatic counting with image analyzers is used, is photo-bleaching of the dye. Dr. Wynn-Williams showed that a bleaching retardant called Citifluor AF2, when used as a microscopic mountant, prevented this problem. In spite of the utility of this modification for epifluorescent counting of microbial populations and my familiarity with the work of many microbial ecologists, I know of no one who uses this technique. In Dr. Wynn-Williams' laboratory, this techniques is used routinely with image analysis in studies of Antarctic microorganisms attached to inorganic particles.

Drs. R.I. Lewis Smith and D.. Wynn-Williams publish annually a complete bibliography of Antarctic terrestrial research called the *Biotas Newsletter*. The newsletter covers Antarctic limnology as well as terrestrial work, and has descriptions of all national programs of research and relevant meetings.

Concluding Remarks

There is strong image analysis expertise here. The BAS makes its impact on U.K. science by its collaborative programs. As one who has been interested in the Antarctic for many years, I found this laboratory a fascinating place to visit. The Antarctic expedition logistical support areas were particularly impressive.

Scotland

The University of Stirling, Natural Environment Research Council Unit for Marine Biochemistry

Introduction

By European standards, the University of Stirling is very new--less than 25 years old. Unusual qualities are: it has always functioned on a semester basis, has highly flexible course arrangements, and stresses cross-disciplinary degrees such as "Business and the Japanese Language" and "Aquaculture." Therefore, it is not unexpected that some of the research carried out at Stirling is also less constrained by disciplinary boundaries than in other institutes.

The research group I visited was the Natural Environmental Research Council (NERC) Unit for Aquatic Biochemistry. The unit was formed from a nucleus of scientists who moved to Stirling from the Institute of Marine Biochemistry in Aberdeen and is housed in modern laboratories. Director Dr. John Sargent explained that the research of the unit is directed towards improving our knowledge of the natural aquatic environment (both fresh water and marine) so that predictive information can be obtained. All the research is at the molecular or cellular level and is focused in two general areas--(1) lipid structure and function and (2) molecular toxicology. The toxicology and lipid groups appear to be unusual in that they are highly committed to both molecular and field programs.

Lipid Structure and Function

Much of the work of this section of the NERC unit centers around polyunsaturated fatty acids (PUFA)--molecules that originate in the phytoplankton but are essential dietary components of higher organisms such as zooplankton, including larval fishes. The research carried out is heavily experimental, but does have field components, notably a collaboration with scientists in Spitsbergen, Norway. A further facet of this concentration on lipids is their use as biomarkers in dissecting marine symbioses and food webs. The latter is particularly important locally since sea lochs in Scotland support a large salmon aquaculture effort, and salmonids have an absolute requirement for (N-3)PUFAs. Currently, these essential nutrients are supplied from fish meal.

Professor Sargent's group is researching other new sources of these valuable molecules for both fish and human nutrition. To this end, they have analyzed the fatty composition of several microalgal species and have researched the means by which the physiological status of the cells influences the results. Until now, their findings have shown that as these cells become older; i.e., when they divide less frequently, the fatty acids of their lipids become more saturated, shorter in chain length, and are found largely in wax esters or triglycerides. Dr. Sargent pointed out that currently the only practical means of enhancing the PUFA content of farmed salmon was by dietary supplements of fish oil rich in PUFA. Functional roles for the highly unsaturated species of these molecules are also being followed, especially at the transmembrane-signaling level. The group is a source of information on the lipids of many marine organisms, especially those in the plankton.

Molecular Toxicology

Another major interest of the NERC unit is that followed by Dr. Steven George's group. They are conducting basic studies of biochemical systems responsible for the detoxification of organic and metal pollutants. The information obtained is expected to aid in the design of molecular probes able to detect levels of pollutant in the environment below those needed to cause lethal effects. Dr. George points out that measurement of primary, sublethal, pollutant response, which precedes the development of overt pathology, can serve as an early warning of potential environmental impact. For instance, levels of toxicants that influence behavior are far lower than those that are needed for mortality. In many cases, pollutant detoxification systems are inducible by sublethal levels of the chemical. Thus, if the products of the induction can be detected, a mechanism to gauge environmental stress can be developed. This is the research strategy that this group has adopted. For instance, in the induction of mixed-function oxidases that add -OH groups to aromatic rings, the situation is complicated by the fact that several forms of the enzyme system (including the cytochrome P-450 moiety) are constitutive in fish liver cells. However, only the levels of some of these isoforms can be increased in response to various pollutants. This, in turn, provides a means of differentiating between the stress-related and the normal mixed-function oxidases. Dr. George explained that although mixed-function oxidases can be regarded primarily as detoxification systems, in some organisms they are responsible for activating xenobiotics; i.e., they convert relatively innocuous components into extremely

toxic ones. This facet of their activity makes them particularly appropriate indicators. Dr. George favors a quantitative immunological (Western Blot) approach rather than using enzymological assays to detect the P-450 system in livers from stressed fish.

Certain heavy metals, as well as organic materials, find their way into our environment and present us with a pollution problem. Dr. George has worked in this area of physiological response to pollution stress. As in organic pollution, certain specific proteins are induced in organisms by metal exposure. Thus, measurement in tissues of these macromolecules, collectively known as metallothioneins, provides a sensitive indicator of stress. For instance, exposure of fish to cadmium or mercury can result in a 20- to 30-fold increase in hepatic metallothionein. Dr. George points out that many proteins will bind metals nonspecifically. Thus, an analysis based totally on the metal burden of an animal does not infer recent metal exposure. What is needed is a specific assay for the induced heavy-metal binding protein. In his laboratory, assays based on a polyclonal antibody reaction or radioactive cadmium binding are used. The antibody, which was prepared from trout, cross reacts with metallothionein from other fish species. This is not surprising since the gene for metallothionein is highly conserved. The assay has been used to show that zinc injections cause a 10- to 20-fold increase in hepatic metallothionein in marine flatfish. Since the Zn-binding protein has a half-life of about 30 days, this assay can detect exposures in the recent past indicating that care is necessary in interpreting analyses from fish obtained from the field.

Since environmental pollution is rarely of one type, it is important to examine the effects of multiple stresses on an organism. Dr. George's group has examined the activities of both mixed function oxidases (organic pollution indicators) and metallothionein levels (for heavy-metal exposure) in salmon from control rivers and rivers where fish are known to be jaundiced. They found that even in fish that showed no obvious pathology, there were elevated P-450 and metallothionein levels. Work in this laboratory continues to dissect the toxic interactions in situations such as this. A major problem is the influence of enzymes induced by concurrent heavy-metal exposure on mixed function oxidases systems. Because of this, the group is developing new procedures that will be helpful in working with multiple pollutant exposures and the interactions they engender. The first is a fish cell culture system (plaice) to replace use of whole fish, and another is an RNA probe for metallothionein.

Concluding Remarks

This laboratory is at the forefront of the new discipline of molecular ecology.

Wales

University College of North Wales, School of Ocean Sciences, Menai Bridge

Introduction

The University College of North Wales (UCNW) is one of six that form the University of Wales, which with 17,000 students, is the second largest in the United Kingdom (U.K.). The Menai Bridge site has 3,500 students 10 percent of which list Welsh as their first language. The site is within minutes of the Snowdonia National Park--a mecca for rock climbers the world over.

The School of Ocean Sciences (School) was founded in 1986 by the amalgamation of the Marine Biology and Physical Oceanography Departments and is now one of two Centers of Excellence in University Oceanography designated by the University Funding Committee (UFC) of the U.K. The UFC oversees basic funding for U.K. universities and is distinct from the research councils such as the National Environment Research Council (NERC). The School is a well integrated, rather traditional marine laboratory. The biological section has made a commitment to promote molecular techniques, but because of insufficient resources and an emphasis on global flux programs, is making slow progress in that direction.

The school is 2 miles from the main part of UNCW in such extremely crowded conditions that on-site expansion is totally impossible. The School is also a part of Marintech North West--a consortium that includes marine researchers at the Universities of Liverpool, Manchester, and Salford and the University of Manchester Institute of Science Technology (UMIST).

The usual marine institution courses are taught at the School, but there are some unusual ones. For instance, a special program for training Indonesian students (M.Sc. & Ph.D.) is available as well as masters degree programs in Applied Oceanography, Environmental Pollution, or Shell Fish Biology and Culture. Undoubtedly, the latter course owes much to Professor Dennis Crisp who did his pioneering work on barnacles at this school. I visited the laboratories of Professor Peter J. LeB. Williams, Drs. George Floodgate, John Latchford, David Holland, and John Turner. One shelf research vessel is available (*Prince Madog*, 29m.) and one nearshore craft (*Lewis Morris*, 14m).

Specific Projects

Several laboratories are worthy of in-depth discussion.

Professor Peter J. LeB. Williams' Laboratory. Professor Williams is one of the U.K.'s best known biological oceanographers. He came to the field late, having trained in biochemistry. He has held positions in the U.S. and was once chairman of the department surveyed here under the heading of "Sweden". After early pioneering work on the role of heterotrophy in the sea, he has turned recently to biogeochemistry. I should emphasize that this change was one where fashion was made, not followed. He is heavily involved in the Global Ocean Flux Study (GOFS), especially where the measurement of total dissolved carbon dioxide (TCO_2) is concerned.

Cooperating with the University of Rhode Island, this group has developed a fully automatic system controlled by a microcomputer. It is possible to make measurements with a frequency of 8 minutes while the ship is underway. At the same time, samples are being taken from an inlet on the underside of the ship's hull. Cruises along the Biogeochemical Ocean Flux Study sampling meridian (20°W) showed close, negative correlation between underway CO_2-related parameters (TCO_2, pCO_2)and chlorophyll. Since three of the four parameters in the dissolved CO_2 equation are measured by this group, they can calculate internal consistence of their results. These parameters are measured to: $TCO_2 \pm 0.3\mu mol.kg^{-1}$, p $CO_2 \pm 1.0$ppm, and pH, ± 0.005 units. They represent considerable improvement over the figures given in the last paper from this group. Using these figures, they estimate errors in alkalinity to be between 0.36 and 0.37 percent, depending on which parameters are used in the calculation. This particular cruise was taken aboard *R.R.S. Discovery* in conjunction with scientists from Plymouth and the Institute of Oceanographic Sciences Deacon Laboratory (NERC). A feature of the results obtained is the large local spatial variations noticed--a situation only detectable with underway continuous sampling and high reproducibility of measurements. Professor Williams believes that the wide range of values quoted for photosynthetic quotients is caused by the use of techniques, the precision of which is considerably less than the one developed by this group and its collaborators. Other interests of this laboratory (in conjunction with colleagues) include relationships between organisms and turbulence and the effects of islands on oceanic mixing.

Drs. George Floodgate and John Latchford's Laboratory. Dr. Floodgate was one of the first modern microbiologists to be given the marine epithet. For instance, he was the mentor of such well-know marine microbiologists as Madilyn Fletcher, U.S., and Karin

Lochte, Federal Republic of Germany (FRG). He will retire soon, and Dr. John Latchford will replace him at UCNW. Dr. Latchford was hired specifically to accelerate marine microbiology's move here towards molecular biology.

With Dr. Floodgate, Mr. Pablo Intriago from Ecuador is investigating the quality of fatty acids in flexibacteria isolated from shrimp ponds in his native country. The bacteria can form a source of essential polyunsaturated fatty acids for shrimp larvae and maybe able to replace algae in their diet. However, the PUFA content of flexibacteria is unstable and is influenced by the environmental parameters. For instance, increases in salinity (Na) cause the amount of PUFA per cell to increase. This stress response is exactly the opposite to the situation found by other workers in Chlorella. Cyclic AMP in the bacterial growth medium inhibits PUFA synthesis, as measured by the incorporation of radiolabelled acetate or palmitate into linolenic acid. Mr. Intriago thinks that flexibacteria have both the aerobic and anaerobic pathways of PUFA synthesis.

In an entirely different field, Dr. Floodgate collaborates with the marine geophysical group at UCNW in trying to define the source of methane in porous rocks that have unusual acoustical signatures.

Dr. John Turner's Laboratory. Dr. Turner is comparatively new at Menai Bridge. His interests are in the physiology of symbiosis and much of his current field work is cited at Lough Hyne, County Cork, Eire--the first marine preserve in Europe. Having grown up (marine biologically speaking) in the subtropics, I was surprised to learn that less than 10 percent of British coastal anthozoa contain algal symbionts. The figure is similar for other temperate, nutrient-rich waters. In tropical associations, larvae are infected with algal from the open water. The rarity of symbiotic species in temperate waters requires that adults have a more reliable means of symbiont transmission to their offspring. Dr. Turner has found that in *Anemonia viridis*, a sea-anemone found locally, algal symbionts (*Symbiodinium microadriaticum*) are acquired directly through maternal inheritance; i.e., they are transferred in the ova. Physiological ecological studies of these organisms in Lough Hyne shows that an autotrophic contribution to host metabolism is possible only during the summer months when irradiance is high.

Dr. David Holland's Laboratory. Dr. Holland is a chemist who has now spent several years working with bioactive lipids and was once a member of the NERC barnacle unit headed by the late Professor Dennis Crisp. He is still interested in invertebrate larval settlement. Recent work has included the isolation and identity of hatching factors for *Elminius* and *Balanus* barnacies, work originated in the 1950s by Crisp's group and recently concluded in conjunction with scientists from the Imperial Chemical Industry's pharmaceutical division. The compounds proved to be mono- and tri-hydroxy eicosanoids and show hatching activity at 10^{-10}M. This type of work will continue under European Community sponsorship but with the emphasis changed to the commercially important scallop. Unfortunately for the department, Dr. Holland's chief colleague, Dr. Elizabeth Hill, is leaving for a position in the U.S.

Dr. Holland described another project in his laboratory as serendipitous! Analytical chemical investigations of oil seeps from a beach led to the discovery of compounds that enhance larval settling (metalloporphyrins) and others that inhibited setting. The inhibitors of settling are being investigated as a proprietary project. No details of the compounds were available other than that they were terpenoid.

Concluding Remarks

This is a small but modern thinking marine institute whose focus is in understanding traditionally studied phenomena with new versions of old techniques. Perhaps we don't all need to be genetically orientated molecular biologists to understand the oceans!

*U.S. Government Printing Office: 1991 — 312-532/40001